

milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0 decimeter polarimeter tube.

(8) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(9) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 45 FR 64568, Sept. 30, 1980; 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

#### § 455.11 Chloramphenicol palmitate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate is the white to grayish-white, tasteless palmitic acid ester of chloramphenicol. It is so purified and dried that:

(i) It contains not less than 555 micrograms nor more than 595 micrograms of chloramphenicol per milligram.

(ii) [Reserved]

(iii) Its melting range is  $91^{\circ} \pm 4^{\circ}$  C.

(iv) Its specific rotation in absolute ethyl alcohol at  $25^{\circ}$  C. is  $+23^{\circ} \pm 2^{\circ}$ .

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for chloramphenicol content, melting range, specific rotation, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—(1) Chloramphenicol content.* Proceed as directed in § 436.335 of this chapter.

(2) [Reserved]

(3) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of sample in a 25-milliliter, glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary to effect solution. Bring the solution to  $25^{\circ}$  C. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 49 FR 6093, Feb. 17, 1984; 50 FR 19921, May 13, 1985]

#### § 455.12a Sterile chloramphenicol sodium succinate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Chloramphenicol sodium succinate is the light-yellow, water-soluble, ethanol-soluble sodium salt of the 3-monosuccinate ester of chloramphenicol. It is so purified and dried that:

(i) Its potency is not less than 650 and not more than 765 micrograms per milligram. If it is packaged for dispensing, its potency when reconstituted as directed in the labeling is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of chloramphenicol per milliliter that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv)—(v) [Reserved]

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 250 milligrams of chloramphenicol per milliliter is not less than 6.4 and not more than 7.0.

(viii) Its specific rotation in an aqueous solution containing 50 milligrams per milliliter at 25° C. is  $+6.5^{\circ} \pm 1.5^{\circ}$ .

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and specific rotation.

(ii) Samples required:

(a) If the batch is packaged for repackaging or for use in the manufacture of another drug:

(1) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(2) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(b) If the batch is packaged for dispensing:

(1) For all tests except sterility: A minimum of 8 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Working standard.* Dissolve an accurately weighed portion of the chloramphenicol working standard in sufficient distilled water to give a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wavelength of 278 nanometers.

(ii) *Procedure.* Dissolve an accurately weighed portion of the sample to be tested in sufficient distilled water to give a solution containing 30 micrograms of the sample per milliliter (estimated); and also if it is packaged for dispensing, reconstitute as directed in the labeling. Remove an accurately measured representative portion from each container and further dilute this portion with sufficient distilled water to give a concentration of 20 micrograms of chloramphenicol per milliliter (estimated). Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wave length of 276 nanometers. Calculate the micrograms per milligram of the dry powder as follows:

$$\text{Micrograms of chloramphenicol per milligram} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{potency of chloramphenicol working standard in the micrograms per milligram}}{\text{Absorbance of standard at 278 nanometers} \times \text{micrograms of sample per milliliter}}$$

Calculate the milligrams per milliliter of the reconstituted solution in the dispensing container as follows:

$$\text{Milligrams per milliliter of the reconstituted vial} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{labeled content of reconstituted vial in milligrams per milliliter}}{\text{Absorbance of standard at 278 nanometers} \times 20}$$

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter.

(4)—(5) [Reserved]

(6) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(7) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of chloramphenicol per milliliter.

(8) *Specific rotation*. Dilute the sample with sufficient distilled water to give a solution containing approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

[39 FR 19166, May 30, 1974, as amended at 39 FR 37486, Oct. 22, 1974; 45 FR 64568, Sept. 30, 1980; 50 FR 1504, Jan. 11, 1985; 50 FR 19921, May 13, 1985]

#### § 455.15 Clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.5 percent.

(iii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(iv) It gives a positive identity test.

(v) Its content of the potassium salt of [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) is satisfactory if it is not greater than .01 percent.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the re-

quirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content*. Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(a) *0.05M Sodium phosphate buffer solution, pH 4.4*. Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4±0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(b) *Mobile phase*. Mix methanol: 0.05*M* sodium phosphate buffer, pH 4.4 (5:95 v/v) and stir or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Preparation of clavulanic acid working standard and sample solutions*. Accurately weigh and transfer into volumetric flasks sufficient clavulanic acid working standard or clavulanate potassium sample to obtain a final concentration of 250 micrograms per milliliter. To the clavulanic acid working standard, add sufficient amoxicillin trihydrate to provide a final concentration of 500 micrograms per milliliter. (The amoxicillin serves as an internal marker for system suitability testing.) Dissolve in water by shaking or